The Benzyl derivative 4 has moderate activity relative to the benzamide derivative 3 as shown in Figure 2.

Finally, the N-acetylation of the benzyl derivative 4 leads to an improvement in the response, but the activity remains lower than that of the benzamide derivative 3 as shown in Figure 3.

These results indicate the importance of the amide bond. The results obtained with these benzamide derivatives confirm the effect of the amide-double bond conjugation present on the natural compound.

The benzamide 7 meta-substitute with the undec-4-ynyloxy chain shows activity comparable to that of the benzamide derivative 3, whereas the benzamide compound 6 substituted with the fully saturated chain shows slightly lower activity. These results indicate that an unsaturation in position 4 may lead to an increase in activity as shown in Figure 4.

The tests relating to the benzamide derivatives 8 and 9 ortho- and para-substituted with the undec-4Z-enyloxy chain reveal analogs that are less active than the meta-substituted benzamide derivative 3. The meta-substitution is thus preferred as mimic for an unsaturation of trans type.

II-7.1.2 Tests of induction of early nodulin on *Medicago truncatula*

These tests are performed to determine whether the synthetic LCOs induce symbiotic responses by activation of the same signal transduction pathway as the natural Nod factors. The tests are performed on the model legume *Medicago truncatula*. The activity of the sulfated benzamide derivative 3, meta-substituted with the undec-4Z-enyloxy chain, which is the most active synthetic compound in the nodulation test on alfalfa, is studied on "wild-type" plants and on a mutant in the gene *DMI1* which is altered in the transduction of the Nod factor signal (Catoira et al. *Plant Cell*, 12, 1647-1665, 2000). The compound that serves as reference is the sulfated tetramer 12 acylated with the C16:2Δ2E,9Z chain, which is an analog of the natural Nod factor. The control is the plant cultivated in the absence of LCO.

II-7.1.2.1 Reporter gene

It is generally difficult to determine the regulation of expression of a particular gene, during a biological process, since most of the specific products of these genes are not readily detectable

responses of two type of transgenic plants bearing the *MtENOD11::GUS:* fusion are compared: a "wild-type" (WT) Jemalong plant and a plant bearing a mutation in the *DMI1* gene, which is incapable of transducing the Nod factor signal. The plants are left to grow for 5 days and the plantlets are then treated with various concentrations of LCO. After 6 hours, the plantlets are removed and placed in aqueous medium containing X-Gluc for 1 to 2 hours. The number of roots giving a characteristic blue response is then counted.

This test is relatively sensitive, to the extent that it is possible to work at LCO concentrations that are lower than those for the modulation tests as shown in Figure 5.

It is found that the benzamide derivative 3 is approximately 10 time less active than the reference compound, the acylated tetramer 12. Moreover, as for the reference compound 12, the benzamide derivative 3 does not induce any response in the plants bearing the DMI1 mutation. It may thus be concluded that the synthetic compound of benzamide type activates the transcription of the ENOD11 gene via the same transduction pathway as that activated by the natural Nod factors.